

**This Page Is Inserted by IFW Operations  
and is not a part of the Official Record**

## **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning documents *will not* correct images,  
please do not report the images to the  
Image Problem Mailbox.**

**THIS PAGE BLANK (USPTO)**

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>7</sup> :</b> <b>A61K 49/00</b>	<b>A2</b>	<b>(11) International Publication Number:</b> <b>WO 00/21576</b> <b>(43) International Publication Date:</b> 20 April 2000 (20.04.00)
<b>(21) International Application Number:</b> PCT/US99/21607 <b>(22) International Filing Date:</b> 12 October 1999 (12.10.99)  <b>(30) Priority Data:</b> 09/173,190 15 October 1998 (15.10.98) US 09/362,805 28 July 1999 (28.07.99) US  <b>(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Applications</b> US 09/173,190 (CIP) Filed on 15 October 1998 (15.10.98) US 09/362,805 (CIP) Filed on 28 July 1999 (28.07.99)  <b>(71) Applicant (for all designated States except US):</b> FLUORO PROBE, INC. [US/US]; 1211 Alameda Boulevard, Coronado, CA 92118 (US).  <b>(72) Inventor; and</b> <b>(75) Inventor/Applicant (for US only):</b> LUIKEN, George, A. [US/US]; 1211 Alameda Boulevard, Coronado, CA 92118 (US).		<b>(74) Agent:</b> LEARN, June, M.; Gray Cary Ware & Freidenrich LLP, Suite 1600, 4365 Executive Drive, San Diego, CA 92121 (US).  <b>(81) Designated States:</b> AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i>
<b>(54) Title:</b> METHOD FOR VIEWING TUMOR TISSUE LOCATED WITHIN A BODY CAVITY  <b>(57) Abstract</b>  Methods are provided for <i>in vivo</i> detection of tissue associated with a disease state in a subject, such as tumor tissue located in a body opening. In the invention method, the subject is administered a biologically compatible fluorescing targeting construct, the construct is allowed to bind to and/or be taken up by diseased tissue present in the subject, a body part of the subject suspected of containing the tumor tissue is irradiated <i>in vivo</i> with UV light while extraneous light to the body part is substantially eliminated, and fluorescence emanating from the fluorescing targeting construct bound to and/or taken up by the tumor tissue is directly viewed by the observer with or without the aid of an endoscope, so as to determine the location and size of the tumor tissue. The invention methods offer the advantage that diseased or abnormal tissue can be directly viewed at interior body sites with or without the aid of an endoscopic device, and without the use of additional imaging equipment, for example, through a surgical opening to facilitate a procedure of biopsy or surgical excision.		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

## METHOD FOR VIEWING TUMOR TISSUE LOCATED WITHIN A BODY CAVITY

### FIELD OF THE INVENTION

The present invention relates to methods for viewing the state of a body cavity or an internal organ of a mammalian body. More particularly, the invention relates to a method for *in vivo* detection of tumor tissue at an interior body site using a  
5 fluorescent targeting construct excited by UV light.

### RELATED APPLICATIONS

This application is a Continuation-In-Part application of copending U. S. Patent Application Serial No. 09/173,190, filed October 15, 1998, entitled "Method For Viewing Diseased Tissue Located Within A Body Cavity," which is incorporated  
10 herein by reference in its entirety.

### BACKGROUND OF THE INVENTION

Many solid and liquid substances naturally emit fluorescent radiation when irradiated with ultraviolet light. However, the radiation may fall within wide wavelength bands of low intensity. In the case of many natural objects, observations  
15 are partially obscured by natural fluorescence emanating simultaneously from many different compounds present in the sample under examination. In imaging devices such as microscopes, therefore, it is known to employ a filter for a selected UV wavelength band to screen out undesired fluorescence emanating from the object under observation.

20 In medical applications, a similar difficulty arises because both tumors and healthy tissue fluoresce naturally, albeit at different wavelengths. Consequently, when UV-activated fluorescence is used to detect tumors against a background of healthy tissue, identification of tumors is difficult. However, unlike most other cells of the body, tumor cells may possess a natural ability to concentrate and retain  
25 hematoporphyrin derivative dyes. Based upon this discovery, a technique was developed wherein a hematoporphyrin derivative fluorescent dye is administered and allowed to concentrate in a tumor to be examined to increase the fluorescence from

the tumor as compared with that of healthy background tissue. Hematoporphyrin dyes fluoresce within a fluorescence spectrum between 610 and 700 nm, a spectrum easy to detect. However, the natural fluorescence from healthy cells is still much more intense than that from the dyes, and has a broader fluorescence spectrum. Thus, the use of fluorescent dyes in diagnosis of tumors has not been wholly successful.

In endoscopic systems, it is also known to irradiate an internal organ with visible radiation to obtain a visible image and then to apply to the internal organ a fluorescent dye that concentrates in tumors over a period of time. The dye is allowed to concentrate, and then the internal organ is irradiated with excitation radiation for the dye to obtain a second fluorescent image. A body part having abnormal or diseased tissue, such as a cancer, may be identified by comparing an image produced by visible radiation of the internal organ with the image produced by fluorescence. To aid in visualizing the images received, endoscopic systems commonly utilize a television camera attached to a fiber optic scope having an optical guide fiber for guiding a beam from an external radiation source to the internal organ, and another optical guide fiber for transmitting a fluorescent image of the affected area to a television monitor for viewing. These two approaches are combined in a method of the type disclosed in U.S. Patent 4,821,117, wherein a fluorescent dye is applied to an object to be inspected, allowed to concentrate in the tumor, and the affected site is then alternately irradiated with visible light and with radiation at the excitation wavelength of the fluorophore. Images of the object obtained independently by visible and fluorescent light using a TV camera are stored in memory, and are simultaneously displayed in a television monitor to visually distinguish the affected area of the body part from the healthy background tissue.

In another type of procedure, such as is described in U. S. Patent No. 4,786,813, a beam-splitting system splits the fluorescence radiation passing through the optical system into at least three parts, each of which forms a respective image of the object corresponding to each of the wavelength regions received. A detector produces a cumulative weighted signal for each image point corresponding to a single point on the object. From the weighted signal values of the various points on the object, an image of the object having improved contrast is produced. This technique

is used to aid in distinguishing the fluorescence from the affected tissue from that produced by normal tissue.

A still more complex method of visualizing images from an endoscopic device uses television scanning apparatus. For example, U. S. Patent No. 4,719,508  
5 discloses a method utilizing an endoscopic photographing apparatus wherein the endoscope includes an image sensor for successively generating image signals fed to a first frame memory for storing the image signals and a second frame memory for interlacing and storing image signals read successively from the first frame memory. The stored, interlaced image signals are delivered to a TV monitor for display to aid  
10 in visualizing the affected body part.

These prior art endoscopic systems, which rely on photographic imaging of the area of interest (i.e., via a TV monitor), while effective, have historically relied on increasingly complex and expensive equipment and substitute indirect viewing for direct viewing of the affected body part.

15 Certain of the fluorescent dyes that concentrate in tumors due to natural bodily processes can be excited at wavelengths corresponding to those produced by lasers to accomplish diagnostic and therapeutic purposes. Consequently, lasers have also been used in procedures utilizing endoscopic systems in conjunction with fluorescent dyes to image and treat tumors. In one embodiment of this general method, a dye is used  
20 that absorbs laser light at two different wavelengths and/or laser powers, one that excites fluorescence without generating damaging heat in the tissue, and one that generates sufficient heat in the dye to destroy surrounding tissue. U.S. Patent No. 4,768,513, for example, discloses a procedure in which a dye is applied to a body part suspected of containing a tumor, usually by local injection. The dye is allowed to  
25 concentrate in tumors and clear from healthy tissue over a period of days, and then the body part is irradiated with alternate pulses of two light sources: a white light of a known intensity and a fluorescence-exciting laser light. To compensate for variations in intensity of the fluorescence resulting from variations in the angle of incident light, and the like, visualization of the tumor is computer-enhanced by calculating the  
30 intensity of the fluorescence with respect to the known intensity of the white light.



Ablation of a tumor detected using this method is accomplished by switching the laser to the heat-generating wavelength so as to destroy the cancerous tissue into which the fluorophore has collected.

While effective for diagnosing and treating tumor, such methods have two major drawbacks. Disease states other than tumor cannot be diagnosed, and laser visualization must be delayed for a period of two days or more after administration of the fluorescent dye to allow the dye to clear from normal tissue.

Monoclonal antibodies and other ligands specific for tumors have been developed for use in diagnosis of tumors, both in tissue samples and in vivo. In addition to such ligands, certain tumor-avid moieties are disproportionately taken up (and optionally or metabolized by tumor cells). Two well-known tumor-avid compounds are deoxyglucose, which plays a telling role in glycolysis in tumor cells, and somatostatin, which binds to and/or is taken up by somatostatin receptors in tumor cells, particularly in endocrine tumors.

In such studies, deoxyglucose is used as a radio-tagged moiety, such as fluorodeoxyglucose ( $^{18}\text{F}$ -deoxyglucose), for detection of tumors of various types. It is believed that tumor cells experience such a mismatch between glucose consumption and glucose delivery that anaerobic glycolysis must be relied upon, thereby elevating the concentration of the radioactive tag in tumor tissue. It is also a possibility that the elevated concentration of deoxyglucose in malignant tumors may be caused by the presence of isoenzymes of hexokinase with abnormal affinities for native glucose or its analogs (A. Gjedde, Chapter 6: "Glucose Metabolism," Principles of Nuclear Medicine, 2<sup>nd</sup> Ed., W. B. Saunders Company, Philadelphia, PA, pages 54-69). Similarly, due to the concentration of somatostatin in tumor tissue, radio-tagged somatostatin, and fragments or analogs thereof, are used in the art for non-invasive imaging of a variety of tumor types in a procedure known as somatostatin receptor scintigraphy (SRS).

Although these techniques have met with considerable success in determining the presence of tumor tissue, scintigraphic techniques are difficult to apply during a surgical procedure because of the equipment necessary for viewing the image



provided by the radioisotope. Yet it is exactly at the time that the surgeon has made the incision or entered the body cavity that it would be most useful to "see" the outlines of the diseased tissue.

Thus, there is a need in the art for new and better methods that can be used to  
5 directly visualize a broad range of putative disease sites using fluorophores that are excited by UV light with or without the use of endoscopic instruments. Where visualization is by means of endoscopic devices, direct visualization (as opposed to creation of photographic images) offers the additional advantage that the equipment required is comparatively simple to use and is less expensive than the equipment  
10 required to create photographic displays from such images. In addition, there is a need in the art for a method of identifying diseased or abnormal tissue during surgical procedures so that immediate resection or biopsy of the identified tissue can be performed while the surgeon "sees" the outlines of the diseased or abnormal tissue.

#### SUMMARY OF THE INVENTION

15 The present invention provides method(s) for *in vivo* identification of tissue associated with a disease state in a subject in need thereof. The invention method(s) comprise administering to the subject a diagnostically effective amount of a fluorescing targeting construct so as to allow the fluorescing targeting construct to bind to and/or be taken up by the target tissue, irradiating a body part of the subject  
20 suspected of containing the target tissue with UV light having at least one wavelength in the excitation spectrum of the targeting construct under conditions that substantially eliminate extraneous light to the body part, and directly viewing *in vivo* fluorescence emanating from the fluorescing targeting construct bound to the target tissue so as to determine the location and/or surface area of the target tissue in the  
25 body part. The targeting construct generally comprises a UV-fluorescing moiety and a ligand moiety that binds to or is taken up by the target tissue. Preferred types of ligand moiety are antibodies and tumor-avid compounds. Tumor-avid compounds that are preferentially "taken up" by tumor cells and can be used as the ligand moiety in the invention targeting constructs generally enter the cells through surface or

nuclear receptors, such as hormone receptors, or through pores, hydrophilic “windows” in the cell lipid bilayer, and the like.

If the putative body part is a body opening of the subject or a surgically produced interior site, an endoscopic device is optionally used to direct the UV light to the body part and to receive fluorescence from the fluorescing targeting construct for direct viewing. Thus, an endoscopic device can aid in detection of tumor tissue associated with a disease state by direct viewing of fluorescence emanating from the tumor-avid ligand attached to or taken up by the target tumor tissue.

In one embodiment of the invention method, the fluorescing targeting construct comprises a biologically compatible UV-sensitive fluorescing moiety and a tumor-avid ligand moiety that preponderantly binds to and/or is taken up by tumor tissue, such as deoxyglucose, somatostatin, a somatostatin receptor-binding peptide, methionine, and the like. Additional tumor-avid ligands are hormones and other compounds which bind to and/or are taken up preferentially by tumor cells, for example by receptors, such as nuclear receptors, expressed by tumor cells. The tumor can be any type for which a specific tumor-avid ligand is known or can be pre-determined using screening procedures described herein.

The invention method is particularly suited to *in vivo* detection of diseased tissue located at an interior site in the subject, such as within a natural body cavity or a surgically created opening, without the need for an endoscopic device. For example, the diseased tissue (e.g., tumor tissue) can be contemporaneously viewed through a surgical opening to facilitate a procedure of biopsy or surgical excision. As the precise location and/or surface area of the diseased tissue are readily determined by the invention diagnostic procedure, the invention method is a valuable guide to the surgeon, who needs to “see” the exact outlines, size, etc. of the mass to be resected as the surgery proceeds.

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a method for *in vivo* identification of tissue associated with a disease state in a subject in need thereof using a fluorophore

sensitive to UV light. The invention method comprises administering to the subject a diagnostically effective amount of a fluorescing targeting construct so as to allow the fluorescing targeting construct to bind to and/or be taken up by the target tissue, irradiating a body part of the subject suspected of containing the target tissue with UV  
5 light having at least one wavelength in the excitation spectrum of the targeting construct under conditions that substantially eliminate extraneous light to the body part, and directly viewing fluorescence emanating from the fluorescing targeting construct bound to or taken up by the targeting tissue so as to determine the location and/or surface area of the target tissue in the body part.

10 If the putative disease site is a natural body cavity or surgically produced interior site, an endoscopic device can be optionally used to deliver the excitation UV light to the site, to receive fluorescence emanating from the site, and to aid in formation of a fluorescent image of the fluorescence from the diseased tissue. For example, a lens in the endoscopic device can be used to focus the detected  
15 fluorescence as an aid in formation of the image. Alternatively, the UV light may be directed into a body cavity or surgical opening by any convenient means and the fluorescent image so produced can be directly visualized by the observer without aid from an endoscope.

In operating rooms it is possible to have an overhead UV light of the desired  
20 optical emitting spectrum. Such a light can be utilized in the practice of the invention merely by turning out the other lights in the operating room (to eliminate extraneous light that would be reflected from the body part under investigation) and shining the UV light into the body cavity or surgically created opening so that the fluorescent image received directly by the eye of the observer (e.g., the surgeon) is predominantly  
25 the fluorescent image emanating from the fluorophore(s) in the field of vision on the selected body part. In any event, to aid in accomplishing the goal of direct visualization by the observer, extraneous light to the body part of interest is substantially eliminated so that light reflected from the body part, other than that from the UV-sensitive fluorescent moieties, is minimized or eliminated. With or without  
30 aid from any type of endoscopic device, the fluorescent image produced by the invention method is such that it can be viewed without aid of a camera, TV monitor,

or other such additional equipment. For example, the diseased or abnormal tissue can be contemporaneously viewed through a surgical opening to facilitate a procedure of biopsy or surgical excision. As the location and/or surface area of the diseased tissue are readily determined by the invention diagnostic procedure, the invention method is  
5 a valuable guide to the surgeon, who needs to know the exact outlines, size, etc. of the mass to be resected as the surgery proceeds.

In one embodiment of the invention method, a single type of fluorescent moiety is relied upon for forming the fluorescence emanating from the irradiated body part. Since certain types of healthy tissue fluoresce naturally, in such a case it is  
10 important to select a fluorescent moiety for the targeting construct that has a predominate excitation wavelength not shared by the healthy tissue found in the body part to be illuminated by the UV light. In this embodiment of the invention, the irradiating UV light from the light source may be monochromatic or polychromatic with at least one irradiating wavelength matched to the predominant excitation  
15 wavelength of the fluorescent targeting construct. By this means, the amount of fluorescence emanating from healthy "background" tissue in the field of vision is minimized.

In alternative embodiments, the invention method may additionally comprise the step of administering to the subject one or more supplemental fluorescing  
20 targeting constructs (e.g., antibodies, or biologically active fragments thereof, having attached fluorophores) that bind to the initial fluorescent targeting construct and/or to each other to enhance the fluorescence emanating from the target tissue. For instance, a fluorescently tagged anti-fluorophore antibody may be administered to bind to any previously administered fluorescently-tagged antibody or tumor-avid molecule. The  
25 purpose of the supplemental fluorescing targeting construct is to increase the intensity of fluorescence from the targeting ligand of the first administered targeting construct and thereby to aid in detection of diseased or abnormal tissue in the body part.

It is generally good practice to allow the targeting construct to bind to and/or be taken up by any targeting tissue that may be present at the site under investigation  
30 and then, before administration of the supplemental fluorescing targeting construct(s),

to substantially remove (e.g., wash) from the body part any unbound targeting construct to maximize the opportunity for fluorescence from the supplemental fluorescing targeting construct(s) to aid in detecting the presence of any target tissue present in the body part. Usually, the supplemental fluorescing targeting constructs are successively administered to build up the fluorescent signal from the target tissue. For example, if the fluorescent targeting construct comprises a humanized IgG monoclonal antibody specific for a breast cancer antigen, the next-administered fluorescing targeting construct may comprise an anti-fluorophore antibody, such as anti-fluorescein, and the third-administered fluorescing targeting construct may comprise an anti-idiotypic antibody. Those of skill in the art will be able to devise combinations of successively administered fluorescing targeting constructs, each of which specifically binds to the targeting construct or to one or more of the earlier administered supplemental fluorescing targeting constructs. It is presently preferred that all of the fluorescing targeting constructs used to identify the target tissue comprise fluorophores that fluoresce within the same wavelength band or at the same wave length as does the initially administered targeting construct (e.g. the UV-sensitive fluorescing moiety in the initially administered targeting construct) to minimize the number of different UV sources that need to be employed to excite simultaneous fluorescence from all of the different targeting constructs used in practice of the invention method.

In yet another embodiment, the invention method further comprises the step of administering to the subject at least one supplemental fluorescent targeting construct (e.g., comprising an antibody, or a biologically active fragment thereof having an attached fluorophore) that specifically binds to or is taken up by normal tissue or constructs in the body part, wherein fluorescence from the supplemental fluorescent targeting construct(s) in response to the irradiating UV light is a different color (i.e., has a different wavelength) than that from the florescent targeting construct that is selected to bind to or be taken up by the target tissue. The difference in the colors of the fluorescence emanating from fluorophores in targeting constructs targeted to normal and to diseased or abnormal target tissue aids the observer in determining the location and size of the target tissue. This embodiment of the invention provides the advantage that any natural fluorescence emanating from normal tissue is obscured by



the fluorescence emanating from fluorophore(s) in supplemental targeting constructs targeted to the normal tissue in the body part. The greater the difference in color between the fluorescence emanating from normal and target tissue, the easier it is for the observer to visualize the outlines and size of the target tissue. For instance,

5 targeting a fluorescing targeting construct comprising a fluorophore producing red light to the target tissue (i.e., abnormal tissue) and a fluorophore producing green light to healthy tissue aids the observer in distinguishing the target tissue from the normal tissue.

The spectrum of UV light used in the practice of the invention method is

10 selected to contain at least one wavelength that corresponds to the predominate excitation wavelength of the targeting construct, or of a biologically compatible UV-sensitive fluorescing moiety contained within the targeting construct. Generally the UV light used in practice of the invention method comprises at least one wavelength of light in the UV wavelength range, for example in the range from about 4 nm to

15 about 400 nm, and preferably in the range from about 340 nm to about 400 nm. The UV light may be monochromatic or polychromatic.

However, when a combination of targeting ligands that fluoresce at different wavelengths is used in practice of the invention, the spectrum of the UV light must be broad enough to provide at least one excitation wavelength for each of the

20 fluorophores used. For example, it is particularly important when fluorophores of different colors are selected to distinguish normal from diseased tissue, that the excitation spectrum of the UV light(s) include excitation wavelengths for the fluorophores targeted to normal and target tissue.

The UV-sensitive fluorescing moiety of the targeting construct or of the

25 supplemental fluorescing targeting ligand(s) can be any chemical or protein moiety that is biologically compatible (e.g., suitable for *in vivo* administration) and which fluoresces in response to light having a wavelength in the UV range selected. Since the targeting ligand is administered to living tissue, biological compatibility includes the lack of substantial toxic effect to the individual in general, and to the target tissue,

30 in particular. Non limiting examples of UV sensitive-fluorophores that can be used in

the practice of the invention include quinine, fluorescein, tetracycline, Allura Red AC dye (also known as D&C Red #40, having a chemical formula: disodium 6-hydroxy-5-(6-methoxy-4-sulphonato m-tolylazo)naphthalene-2-sulphonate), phloxine B (also known as Acid red 92 or D&C Red 28, having a chemical formula:

5 spiro[isobenzofuran-1(3H),9'[9H]xanthen]-3-one, 2',4'5',7'-tetrabromo-4,5,6,7-tetrachloro-3',6'-dihydroxy-disodium salt), and the like, and combinations of two or more thereof.

Since the fluorescence properties of biologically compatible fluorophores are well known, or can be readily determined by those of skill in the art, the skilled  
10 practitioner can readily select a useful fluorophore or useful combinations of fluorophores, and match the wavelength(s) of UV light to the fluorophore(s). Toxicity of additional useful fluorophores can be determined using animal studies as known in the art.

Preferably, the targeting construct (e.g., the ligand moiety of the invention  
15 targeting construct) is selected to bind to and/or be taken up specifically by the target tissue of interest, for example to an antigen or other surface feature contained on or within a cell that characterizes a disease or abnormal state in the target tissue. As in other diagnostic assays, it is desirable for the targeting construct to bind to or be taken up by the target tissue selectively or to an antigen associated with the disease or  
20 abnormal state; however, targeting constructs containing ligand moieties that also bind to or are taken up by healthy tissue or cell structures can be used in the practice of the invention method so long as the concentration of the antigen in the target tissue or the affinity of the targeting construct for the target tissue is sufficiently greater than for healthy tissue in the field of vision so that a fluorescent image representing the  
25 target tissue can be clearly visualized as distinct from any fluorescence coming from healthy tissue or structures in the field of vision. For example, colon cancer is often characterized by the presence of carcinoembryonic antigen (CEA), yet this antigen is also associated with certain tissues in healthy individuals. However, the concentration of CEA in cancerous colon tissue is often greater than is found in  
30 healthy tissue, so an anti-CEA antibody could be used as a ligand moiety in the practice of the invention. In another example, deoxyglucose is taken up and utilized



by healthy tissue to varying degrees, yet its metabolism in healthy tissues, except for certain known organs, such as the heart, is substantially lower than in tumor. The known pattern of deoxyglucose consumption in the body can therefore be used to aid in determination of those areas wherein unexpectedly high uptake of deoxyglucose  
5 signals the presence of tumor cells.

In one embodiment according to the present invention, the disease or abnormal state detected by the invention method can be any type characterized by the presence of a known target tissue for which a specific binding ligand is known, for example an antibody or tumor-avid moiety. For example, various heart conditions are  
10 characterized by production of necrotic or ischemic tissue or production of arteriosclerotic tissue for which specific binding ligands are known. As another illustrative example, breast cancer is characterized by the production of cancerous tissue identified by monoclonal antibodies to CA15-3, CA19-9, CEA, or HER2/neu. It is contemplated that the target tissue may be characterized by cells that produce  
15 either a surface antigen for which a binding ligand is known, or an intracellular marker (i.e. antigen), since many targeting constructs penetrate the cell membrane. Representative disease states that can be identified using the invention method include such various conditions as different types of tumors, bacterial, fungal and viral infections, and the like. As used herein "abnormal" tissue includes precancerous  
20 conditions, necrotic or ischemic tissue, and tissue associated with connective tissue diseases, and auto-immune disorders, and the like. Examples of the types of target tissue suitable for examination using the invention method include cardiac, breast, ovarian, uterine, lung, endothelial, vascular, gastro-intestinal, colorectal, prostatic tissue, endocrine tissue, and the like, as well as combinations of any two or more  
25 thereof.

Representative examples of antigens for some common malignancies and the body locations in which they are commonly found are shown in Table I below. Targeting ligands, such as antibodies, for these antigens are known in the art.

TABLE I

<u>ANTIGEN</u>	<u>TUMORS WHERE COMMONLY FOUND</u>
CEA (carcinoembryonic antigen)	colon, breast, lung
PSA (prostate specific antigen)	prostate cancer
CA-125	ovarian cancer
CA 15-3	breast cancer
CA 19-9	breast cancer
HER2/neu	breast cancer
$\alpha$ -feto protein	testicular cancer, hepatic cancer
$\beta$ -HCG (human chorionic gonadotropin)	testicular cancer, choriocarcinoma
MUC-1	breast cancer
Estrogen receptor	breast cancer, uterine cancer
Progesterone receptor	breast cancer, uterine cancer
EGFr (epidermal growth factor receptor	bladder cancer

In one embodiment of the invention method, the ligand moiety of the targeting construct is a protein or polypeptide, such as an antibody, or biologically active fragment thereof, preferably a monoclonal antibody. The supplemental fluorescing targeting construct(s) used in practice of the invention method may also be or  
5 comprise polyclonal or monoclonal antibodies tagged with a fluorophore. The term "antibody" as used in this invention includes intact molecules as well as functional fragments thereof, such as Fab, F(ab')<sub>2</sub>, and Fv that are capable of binding the epitopic determinant. These functional antibody fragments retain some ability to  
10 selectively bind with their respective antigen or receptor and are defined as follows:

(1) Fab, the fragment which contains a monovalent antigen-binding fragment of an antibody molecule, can be produced by digestion of whole antibody with the enzyme papain to yield an intact light chain and a portion of one heavy chain;

(2) Fab', the fragment of an antibody molecule that can be obtained by  
15 treating whole antibody with pepsin, followed by reduction, to yield an intact light

chain and a portion of the heavy chain; two Fab' fragments are obtained per antibody molecule;

(3) (Fab')<sub>2</sub>, the fragment of the antibody that can be obtained by treating whole antibody with the enzyme pepsin without subsequent reduction; F(ab')<sub>2</sub> is a dimer of two Fab' fragments held together by two disulfide bonds;

(4) Fv, defined as a genetically engineered fragment containing the variable region of the light chain and the variable region of the heavy chain expressed as two chains; and

(5) Single chain antibody ("SCA"), a genetically engineered molecule containing the variable region of the light chain and the variable region of the heavy chain, linked by a suitable polypeptide linker as a genetically fused single chain molecule.

Methods of making these fragments are known in the art. (See for example, Harlow & Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, New York, 1988, incorporated herein by reference). As used in this invention, the term "epitope" means any antigenic determinant on an antigen to which the paratope of an antibody binds. Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics.

Antibody fragments of the present invention can be prepared by proteolytic hydrolysis of the antibody or by expression in *E. coli* of DNA encoding the fragment. Antibody fragments can be obtained by pepsin or papain digestion of whole antibodies by conventional methods. For example, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted F(ab')<sub>2</sub>. This fragment can be further cleaved using a thiol reducing agent, and optionally a blocking group for the sulfhydryl groups resulting from cleavage of disulfide linkages, to produce 3.5S Fab' monovalent fragments. Alternatively, an enzymatic cleavage using pepsin produces two monovalent Fab' fragments and an Fc

fragment directly. These methods are described, for example, by Goldenberg, U.S. patents No. 4,036,945 and No. 4,331,647, and references contained therein, which patents are hereby incorporated in their entireties by reference. See also Nisonhoff *et al.*, *Arch. Biochem. Biophys.* 89:230, 1960; Porter, *Biochem. J.* 73:119, 1959;

5 Edelman *et al.*, , Methods in Enzymology, Vol. 1, page 422 Academic Press, 1967; and Coligan *et al.* at sections 2.8.1-2.8.10 and 2.10.1-2.10.4. Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical, or genetic techniques may also be used, so long as the fragments bind to the antigen

10 that is recognized by the intact antibody.

Fv fragments comprise an association of  $V_H$  and  $V_L$  chains. This association may be noncovalent, as described in Inbar *et al.*, *Proc. Nat'l Acad. Sci. USA* 69:2659, 1972. Alternatively, the variable chains can be linked by an intermolecular disulfide bond or cross-linked by chemicals such as glutaraldehyde. See, e.g., Sandhu, *supra*.

15 Preferably, the Fv fragments comprise  $V_H$  and  $V_L$  chains connected by a peptide linker. These single-chain antigen binding proteins (sFv) are prepared by constructing a structural gene comprising DNA sequences encoding the  $V_H$  and  $V_L$  domains connected by an oligonucleotide. The structural gene is inserted into an expression vector, which is subsequently introduced into a host cell such as *E. coli*. The

20 recombinant host cells synthesize a single polypeptide chain with a linker peptide bridging the two V domains. Methods for producing sFvs are described, for example, by Whitlow *et al.*, Methods: a Companion to Methods in Enzymology, 2: 97, 1991; Bird *et al.*, *Science* 242:423-426, 1988; Pack *et al.*, *Bio/Technology* 11:1271-77, 1993; Sandhu, *supra*, and Ladner *et al.*, U.S. patent No. 4,946,778, which is hereby

25 incorporated by reference in its entirety.

Another form of an antibody fragment is a peptide coding for a single complementarity-determining region (CDR). CDR peptides ("minimal recognition units") can be obtained by constructing genes encoding the CDR of an antibody of interest. Such genes are prepared, for example, by using the polymerase chain

30 reaction to synthesize the variable region from RNA of antibody-producing cells.

See, for example, Larrick *et al.*, Methods: a Companion to Methods in Enzymology, 2: 106, 1991.

Antibodies which bind to a tumor cell or other disease-associated antigen can be prepared using an intact polypeptide or biologically functional fragment containing small peptides of interest as the immunizing antigen. The polypeptide or a peptide used to immunize an animal (derived, for example, from translated cDNA or chemical synthesis) can be conjugated to a carrier protein, if desired. Commonly used carriers that are chemically coupled to the peptide include keyhole limpet hemocyanin (KLH), thyroglobulin, bovine serum albumin (BSA), and tetanus toxoid, and the like. The coupled peptide is then used to immunize the animal (e.g., a mouse, a rat, or a rabbit).

The preparation of such monoclonal antibodies is conventional. See, for example, Kohler & Milstein, *Nature* 256:495, 1975; Coligan *et al.*, sections 2.5.1-2.6.7; and Harlow *et al.*, in: Antibodies: a Laboratory Manual, page 726 (Cold Spring Harbor Pub., 1988), which are hereby incorporated by reference. Briefly, monoclonal antibodies can be obtained by injecting mice with a composition comprising an antigen, verifying the presence of antibody production by removing a serum sample, removing the spleen to obtain B lymphocytes, fusing the B lymphocytes with myeloma cells to produce hybridomas, cloning the hybridomas, selecting positive clones that produce antibodies to the antigen, and isolating the antibodies from the hybridoma cultures. Monoclonal antibodies can be isolated and purified from hybridoma cultures by a variety of well-established techniques. Such isolation techniques include affinity chromatography with Protein-A Sepharose, size-exclusion chromatography, and ion-exchange chromatography. See, for example, Coligan *et al.*, sections 2.7.1-2.7.12 and sections 2.9.1-2.9.3; Barnes *et al.*, Purification of Immunoglobulin G (IgG), in: Methods in Molecular Biology, Vol. 10, pages 79-104 (Humana Press, 1992).

Antibodies of the present invention may also be derived from subhuman primate antibodies. General techniques for raising therapeutically useful antibodies in baboons can be found, for example, in Goldenberg *et al.*, International Patent Publication WO 91/11465 (1991) and Losman *et al.*, 1990, *Int. J. Cancer* 46:310,

which are hereby incorporated by reference. Alternatively, a therapeutically useful antibody may be derived from a "humanized" monoclonal antibody. Humanized monoclonal antibodies are produced by transferring mouse complementarity determining regions from heavy and light variable chains of the mouse immunoglobulin into a human variable domain, and then substituting human residues in the framework regions of the murine counterparts. The use of antibody components derived from humanized monoclonal antibodies obviates potential problems associated with the immunogenicity of murine constant regions. General techniques for cloning murine immunoglobulin variable domains are described, for example, by Orlandi *et al.*, *Proc. Nat'l Acad. Sci. USA* 86:3833, 1989, which is hereby incorporated in its entirety by reference. Techniques for producing humanized monoclonal antibodies are described, for example, by Jones *et al.*, *Nature* 321:522, 1986; Riechmann *et al.*, *Nature* 332:323, 1988; Verhoeyen *et al.*, *Science* 239:1534, 1988; Carter *et al.*, *Proc. Nat'l Acad. Sci. USA* 89:4285, 1992; Sandhu, *Crit. Rev. Biotech.* 12:437, 1992; and Singer *et al.*, *J. Immunol.* 150:2844, 1993, which are hereby incorporated by reference.

It is also possible to use anti-idiotypic technology to produce monoclonal antibodies which mimic an epitope. For example, an anti-idiotypic monoclonal antibody made to a first monoclonal antibody will have a binding domain in the hypervariable region which is the "image" of the epitope bound by the first monoclonal antibody.

In one embodiment of the invention method, the ligand moiety in the fluorescent targeting construct used in practice of the invention is selected from among the many biologically compatible tumor-avid compounds that bind with specificity to receptors and/or are preferentially taken up by tumor cells. and can be used as the ligand moiety in the invention targeting constructs. Tumor-avid compounds that are preferentially "taken up" by tumor cells may enter the cells through surface or nuclear receptors (e.g., hormone receptors), pores, hydrophilic "windows" in the cell lipid bilayer, and the like.



Illustrative of this class of tumor-avid compounds are somatostatin, somatostatin receptor-binding peptides, deoxyglucose, methionine, and the like. Particularly useful somatostatin receptor-binding peptides are a long-acting, octapeptide analog of somatostatin, known as octreotide (D-phenylalanyl-L-cysteinyl-L-phenylalanyl-D-tryptophyl-L-lysyl-L-threonyl-N-[2-hydroxy-1-(hydroxymethyl)propyl]-L-cysteinamide cyclic (2→7)-disulfide), lanreotide, an oral formulation of octreotide, P829, P587, and the like. Somatostatin-binding peptides are disclosed in U.S. Patent No. 5,871,711, and methods for linking such peptides covalently to a radioisotope through their carboxyl terminal amino acid under reducing conditions are disclosed in U.S. Patent No. 5,843,401, which are both incorporated herein by reference in their entireties. One of skill in the art can readily adapt such teachings for the preparation of UV sensitive somatostatin receptor-binding peptides by substituting the UV-sensitive fluorescing moieties of this invention in the place of a radioisotope.

Somatostatin and somatostatin receptor-binding peptides are particularly effective for use as the tumor-avid ligand moiety in the targeting construct in the invention diagnostic procedures when the disease state is a neuroendocrine or endocrine tumor. Examples of neuroendocrine tumors that can be diagnosed using the invention method include adenomas (GH-producing and TSH-producing), islet cell tumors, carcinoids, undifferentiated neuroendocrine carcinomas, small cell and non small cell lung cancer, neuroendocrine and/or intermediate cell carcinomas, neuroendocrine tumors of ovary, cervix, endometrium, breast, kidney, larynx, paranasal sinuses, and salivary glands, meningiomas, well differentiated glia-derived tumors, pheochromocytomas, neuroblastomas, ganglioneuro(blasto)mas, paragangliomas, papillary, follicular and medullary carcinomas in thyroid cells, Merkel cell carcinomas, and melanomas, as well as granulomas and lymphomas. These tumor cells are known to have somatostatin receptors and can be targeted using somatostatin or somatostatin receptor binding peptides as the tumor-avid ligand in the invention fluorescent targeting construct.

Vasointestinal peptide (VIP), which is used in VIP receptor scintigraphy (I. Virgolini, *Eur J. Clin. Invest.* 27(10):793-800, 1997, is also useful in the invention



method for diagnosis of small primary adenocarcinomas, liver metastases and certain endocrine tumors of the gastrointestinal tract.

Another molecule illustrative of the tumor-avid ligands that are preferentially taken up by tumors is deoxyglucose, which is known to be preferentially taken up in a variety of different types of tumors. Illustrative of the types of tumors that can be detected using deoxyglucose as the tumor-avid ligand moiety in the fluorescent targeting construct as disclosed herein include Preferred tumor targets for deoxyglucose include melanoma, colorectal and pancreatic tumors, lymphoma (both HD and NHL), head and neck tumors, myeloma, cancers of ovary, cancer, breast, and brain (high grade and pituitary adenomas), sarcomas (grade dependent), hepatoma, testicular cancer, thyroid (grade dependent) small cell lung cancer, bladder and uterine cancer, and the like.

Yet other tumor-avid compounds that can be used as the targeting ligand in an invention fluorescing targeting construct are 1-amino-cyclobutane-1-carboxylic acid and L-methionine. L-methionine is an essential amino acid that is necessary for protein synthesis. It is known that malignant cells have altered methionine metabolism and require an external source of methionine.

Additional examples of biologically compatible tumor-avid compounds that bind with specificity to tumor receptors and/or are preferentially taken up by tumor cells include mammalian hormones, particularly sex hormones, neurotransmitters, and compounds expressed by tumor cells to communicate with each other that are preferentially taken up by tumor cells, such as novel secreted protein constructs arising from chromosomal aberrations, such as transfers or inversions within the clone.

The term hormone is used herein to refer to compounds that are expressed within a mammal for action at a remote location and includes such compounds as sex hormones, cell growth hormones, cytokines, endocrine hormones, erythropoietin, and the like. As is known in the art, a number of tumor types express receptors for hormones, for example, estrogen, progesterone, androgens, such as testosterone, and the like. Such hormones are preferentially taken up by tumor cells, for example, via

specific receptors. It is also known in the art that the particular type of receptors expressed by a tumor cell may change over time with the same cell or cell mass, for example, expressing estrogen receptors at one point in time and with the estrogen receptors being substantially replaced with androgen receptors at another point in time.

Therefore, in another embodiment according to the present invention, the invention diagnostic method comprises prescreening of target tumor cells to determine which receptors are currently being expressed by the target cells. In this embodiment, the invention diagnostic method comprises contacting sample(s) of tumor cells obtained from a subject *in vitro* with a plurality of detectably labeled tumor-avid compounds, and determining which of the tumor-avid compounds bind to or are taken up by the sample cells. The invention diagnostic method further comprises administering to the subject a diagnostically effective amount of one or more biologically compatible fluorescing targeting constructs, each comprising as ligand moiety at least one of the tumor-avid compounds determined to bind to and/or be taken up by the tumor cells so as to allow the fluorescing targeting construct to bind to and/or be taken up selectively *in vivo* by tumor tissue, irradiating an *in vivo* body part of the subject suspected of containing the tumor tissue with UV light having at least one wavelength in the excitation spectrum of the targeting construct under conditions that substantially eliminate extraneous light to the *in vivo* body part, and directly viewing fluorescence emanating from the fluorescing targeting construct bound to or taken up by the tumor tissue so as to determine the location and/or surface area of the tumor tissue in the *in vivo* body part. Of course, if the tests determine that the tumor cells are concurrently taking up more than one tumor-avid compound in substantial proportion (e.g., both estrogen and progesterone), the more than one tumor avid compound so determined can be used as the tumor-avid ligand moieties in the targeting constructs in the invention diagnostic method.

Methods for obtaining test tumor cells for prescreening to determine the type(s) of tumor-avid compounds that are currently being taken up (e.g., by specific receptors expressed by the tumor cells) are well known in the art. For example, such

techniques as fine needle aspirates, scrapings, excisional biopsies, and the like, can in many instances be utilized to obtain test tumor cells relatively non-invasively.

*In vitro* tests useful for determining the tumor-avid compounds that are being taken up by test tumor cells are numerous and also well known in the art. Such *in vitro* tests generally involve either sequentially or simultaneously contacting the test cells with a plurality of different tumor-avid compounds. For example, the test cells can be contacted with a panel or library of detectably labeled hormones and/or other known tumor-avid compounds to determine which of the detectably labeled compounds bind to and/or are taken up by the test cells.

In the practice of the present invention, the UV-sensitive fluorescent moiety is linked to the tumor-avid compound used as the ligand moiety in the targeting construct by any method presently known in the art for attaching two moieties, so long as the attachment of the linker moiety to the ligand moiety does not substantially impede binding of the targeting construct to the target tissue and/or uptake by the tumor cells, for example, to a receptor on a cell. Those of skill in the art will know how to select a ligand/linker pair that meets this requirement. For example, with regard to octreotide, it has been shown that coupling of a linker to Tyr3 or Phe1 of octreotide does not prevent the internalization of octreotide after binding to the somatostatin receptor (L.J. Hofland *et al.*, *Proc. Assoc. Am. Physicians* 111:63-9, 1999). It is also known that 1-amino-cyclobutane-1-carboxylic acid can be tagged at the 3 carbon of the ring.

The length of the optional linker moiety is chosen to optimize the kinetics and specificity of ligand binding, including any conformational changes induced by binding of the ligand moiety to a target, such as an antigen or receptor. The linker moiety should be long enough and flexible enough to allow the ligand moiety and the target to freely interact and not so short as to cause steric hindrance between the proteinaceous ligand moiety and the target.

In one embodiment, the linker moiety is a heterobifunctional cleavable cross-linker, such as N-succinimidyl (4-iodoacetyl)-aminobenzoate; sulfosuccinimidyl(4-iodoacetyl)-aminobenzoate; 4-succinimidyl-oxycarbonyl- $\alpha$ -(2-pyridyldithio) toluene ;

sulfosuccinimidyl-6-[ $\alpha$ -methyl- $\alpha$ -(pyridyldithiol)-toluamido] hexanoate; N-succinimidyl-3-(-2-pyridyldithio)-propionate; succinimidyl-6-[3-(-2-pyridyldithio)-propionamido] hexanoate; sulfosuccinimidyl-6-[3-(-2-pyridyldithio)-propionamido] hexanoate; 3-(2-pyridyldithio)-propionyl hydrazide, Ellman's reagent,  
5 dichlorotriazinic acid, S-(2-thiopyridyl)-L-cysteine, and the like. Further bifunctional linking compounds are disclosed in U.S. Patent Nos. 5,349,066, 5,618,528, 4,569,789, 4,952,394, and 5,137,877, each of which is incorporated herein by reference in its entirety.

These chemical linkers can be attached to purified ligands using numerous  
10 protocols known in the art, such as those described in Pierce Chemicals "Solutions, Cross-linking of Proteins: Basic Concepts and Strategies," Seminar #12, Rockford, IL.

In another embodiment presently preferred, the linker moiety is a peptide having from about 2 to about 60 amino acid residues, for example from about 5 to  
15 about 40, or from about 10 to about 30 amino acid residues. This alternative is particularly advantageous when the ligand moiety is proteinaceous. For example, the linker moiety can be a flexible spacer amino acid sequence, such as those known in single-chain antibody research. Examples of such known linker moieties include GGGGS (SEQ ID NO:1), (GGGGS)<sub>n</sub> (SEQ. ID NO:2), GKSSGSGSESKE (SEQ ID  
20 NO:3), GSTSGSGKSSEGKKG (SEQ. ID NO:4), GSTSGSGKSSEGSGSTKG (SEQ ID NO:5), GSTSGSGKSSEGKKG (SEQ ID NO:6), GSTSGSGKPGSGEGSTKG (SEQ ID NO:7), EGKSSGSGSESKEF (SEQ ID NO:8), SRSSG (SEQ. ID NO:9), SGSSC (SEQ ID NO:10), and the like. A *Diphtheria* toxin trypsin sensitive linker having the sequence AMGRSGGGCAGNRVGSSLSCGGLNLQAM (SEQ ID  
25 NO:11) is also useful. Alternatively, the peptide linker moiety can be VM or AM, or have the structure described by the formula: AM(G<sub>2 to 4</sub>S)<sub>x</sub>QAM wherein Q is selected from any amino acid and X is an integer from 1 to 11 (SEQ ID NO:12). Additional linking moieties are described, for example, in Huston *et al.*, *PNAS* 85:5879-5883, 1988; Whitlow, M., *et al.*, *Protein Engineering* 6:989-995, 1993; Newton *et al.*,  
30 *Biochemistry* 35:545-553, 1996; A. J. Cumber *et al.*, *Bioconj. Chem.* 3:397-401, 1992;

Ladurner *et al.*, *J. Mol. Biol.* 273:330-337, 1997; and U.S. Patent. No. 4,894,443, the latter of which is incorporated herein by reference in its entirety.

The targeting constructs and supplemental targeting constructs used in practice of the invention method can be administered by any route known to those of skill in the art, such as topically, intraarticularly, intracisternally, intraocularly, intraventricularly, intrathecally, intravenously, intramuscularly, intraperitoneally, intradermally, intratracheally, intracavitarily, and the like, as well as by any combination of any two or more thereof.

The most suitable route for administration will vary depending upon the disease state to be treated, or the location of the suspected condition or tumor to be diagnosed. For example, for treatment of inflammatory conditions and various tumors, local administration, including administration by injection directly into the body part to be irradiated by UV light (e.g., intracavitarily) provides the advantage that the targeting construct (e.g., fluorescently tagged antibodies) can be administered in a high concentration without risk of the complications that may accompany systemic administration thereof.

The targeting construct is administered in a "diagnostically effective amount." An effective amount is the quantity of a targeting construct necessary to aid in direct visualization of any target tissue located in the body part under investigation in a subject. A "subject" as the term is used herein is contemplated to include any mammal, such as a domesticated pet, farm animal, or zoo animal, but preferably is a human. Amounts effective for diagnostic use will, of course, depend on the size and location of the body part to be investigated, the affinity of the targeting construct for the target tissue, the type of target tissue, as well as the route of administration. Local administration of the targeting construct will typically require a smaller dosage than any mode of systemic administration, although the local concentration of the targeting construct may, in some cases, be higher following local administration than can be achieved with safety upon systemic administration.

Since individual subjects may present a wide variation in severity of symptoms and each targeting construct has its unique diagnostic characteristics,



including, affinity of the targeting construct for the target, rate of clearance of the targeting construct by bodily processes, the properties of the fluorophore contained therein, and the like, the skilled practitioner will weigh the factors and vary the dosages accordingly.

5           The invention composition can also be formulated as a sterile injectable suspension according to known methods using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1-4, butanediol. Sterile, fixed oils are  
10       conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed, including synthetic mono- or diglycerides, fatty acids (including oleic acid), naturally occurring vegetable oils like sesame oil, coconut oil, peanut oil, cottonseed oil, etc., or synthetic fatty vehicles like ethyl oleate, or the like. Buffers, preservatives, antioxidants, and the like, can be  
15       incorporated as required, or, alternatively, can comprise the formulation.

          The invention fluorescing targeting constructs can be produced by well known techniques. For example, well known techniques of protein synthesis can be used to obtain proteinaceous components of the targeting construct if the amino acid sequence of the component is known, or the sequence can first be determined by well known  
20       methods, if necessary. Some of the ligand genes are now commercially available. An advantage of obtaining commercially available genes is that they have generally been optimized for expression in *E. coli*. A polynucleotide encoding a protein, peptide or polynucleotide of interest, can be produced using DNA synthesis technology. Methods for obtaining the DNA encoding an unavailable gene and expressing a gene product  
25       therefrom are well known and will not be described here in detail.

          A fluorescent targeting construct comprising a proteinaceous ligand moiety, a proteinaceous linker moiety, and a proteinaceous fluorophore can also be produced as a fusion protein using well known techniques wherein a host cell is transfected with an expression vector containing expression control sequences operably linked to a  
30       nucleic acid sequence coding for the expression of the fusion protein (*Molecular*

*Cloning A Laboratory Manual*, Sambrook *et al.*, eds., 2nd Ed., Cold Spring Harbor Laboratory, N.Y., 1989).

“Peptide” and/or “polypeptide” means a polymer in which the monomers are amino acid residues which are joined together through amide bonds, alternatively  
5 referred to as a polypeptide. When the amino acids are alpha-amino acids, either the L-optical isomer or the D-optical isomer can be used, the L-isomers being preferred. Additionally, unnatural amino acids such as beta-alanine, phenylglycine, and homoarginine are meant to be included. Commonly encountered amino acids that are not gene-encoded can also be used in the present invention, although preferred amino  
10 acids are those that are encodable. For a general review, see, for example, Spatola, A.F., in *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*, B. Weinstein, ed., Marcel Dekker, New York, p. 267, 1983.

It will be apparent to those skilled in the art that various changes may be made in the invention without departing from the spirit and scope thereof, and therefore, the  
15 invention encompasses embodiments in addition to those specifically disclosed in the specification, but only as indicated in the appended claims.



## WHAT IS CLAIMED IS:

1. A method for *in vivo* identification of tumor tissue associated with a disease state in a subject in need thereof, said method comprising:

5 administering to the subject a diagnostically effective amount of at least one biologically compatible fluorescing targeting construct so as to allow the fluorescing targeting construct to bind to and/or be taken up selectively *in vivo* by tissue associated with a disease state,

10 irradiating an *in vivo* body part of the subject suspected of containing the tumor tissue with UV light having at least one wavelength in the excitation spectrum of the targeting constructs under conditions that substantially eliminate extraneous light to the *in vivo* body part, and

directly viewing fluorescence emanating from the fluorescing targeting construct bound to or taken up by the tissue so as to determine the location and/or surface area of the tissue in the body part.

2. The method according to claim 1 wherein the targeting construct comprises a biologically compatible UV-sensitive fluorescing moiety and a ligand moiety that preponderantly binds to or is taken up by the tissue associated with a disease state.

3. The method according to claim 2 wherein the tissue is tumor tissue and the ligand moiety is a tumor-avid moiety selected from a hormone, a hormone receptor binding-peptide, deoxyglucose, somatostatin, a somatostatin receptor-binding peptide, or a combination of any two or more thereof.

4. The method according to claim 3 wherein the tumor-avid moiety is somatostatin or a somatostatin receptor-binding peptide.

5. The method according to claim 4 wherein the tumor tissue is an neuroendocrine or endocrine tumor.

6. The method according to claim 4 wherein the tumor tissue is melanoma, insulinoma, pancreatic tumors, small cell and non-small cell lung cancer, lymphoma, or ovarian, pituitary, pancreas, or adrenal cancer, brain tumor, colorectal cancer, cutaneous melanoma, epithelial cancer, lung carcinoma, testicular germ cell tumor, or breast cancer.

7. The method according to claim 4 wherein the somatostatin receptor-binding peptide is octreotide, lanreotide, P587 or P829.

8. The method according to claim 3 wherein the targeting ligand is deoxyglucose.

9. The method according to claim 8 wherein disease state is brain tumor, colorectal cancer, lymphoma, cutaneous melanoma, epithelial tumors, lung carcinoma, testicular germ cell tumor, or breast cancer.

10. The method according to claim 2 wherein the ligand moiety is 1-amino-cyclobutane-1-carboxylic acid or methionine.

12. The method according to claim 2 wherein the ligand moiety comprises a monoclonal antibody, or a biologically active fragment thereof, that is specific for an antigen predominantly associated with the disease state.

13. The method according to claim 12 wherein the antigen is selected from the group consisting of cell surface and intracellular antigens and the target tissue is selected from normal, abnormal, and malignant tissue.

14. The method according to claim 12 wherein the antigen is selected from the group consisting of carcinoembryonic antigen, human epithelial and mesothelial cancer antigens, tumor specific glycoproteins, mucin-type carbohydrate chain antigens, PSA, CA-125, CA-15-3, CA 19-9, HER2/neu,  $\alpha$ -feto protein,  $\beta$ -HCG, estrogen receptor antigens, progesterone receptor antigens, and EGFr.

15. The method according to claim 12 wherein the antibody is specific for colon, breast, or ovarian cancer.

16. The method according to claim 12 wherein the antibody is selected from antibodies to CEA, PSA, CA-125, CA 15-3, CA 19-9, HER2/neu,  $\alpha$ -feto protein,  $\beta$ -HCG, MUC-1, estrogen receptor, progesterone receptor, or EGFr.

17. The method according to claim 12 wherein the antibody is specific for an antigen selected from the group consisting of bacterial, fungal, and viral antigens.

18. The method according to claim 1 wherein the target tissue is selected from the group consisting of cardiac, breast, ovarian, uterine, lung, endothelial, vascular, gastro-intestinal, colorectal, prostatic tissue and endocrine tissue.

19. The method according to claim 1 wherein the *in vivo* body part is located in a body opening of the subject and wherein the irradiating of the body part and the viewing of fluorescence involves use of an endoscopic device to direct the UV light to the body part, and to receive fluorescence from the fluorescing targeting  
5 construct.

20. The method according to claim 1 wherein the method further comprises surgically excising at least a part of the tissue while viewing the fluorescent image.

21. The method according to claim 1 wherein the surface area determined is based on the intensity of the fluorescence.

22. The method according to claim 1 wherein the UV light is substantially monochromatic and the wavelength is matched to a predominant excitation wavelength of the fluorescent targeting construct.

23. The method according to claim 1 wherein the at least one wavelength of UV light is in the range from about 4 nm to about 400 nm.

24. The method according to claim 19 wherein the opening is a natural body cavity.

25. The method according to claim 19 wherein the opening is made surgically.

26. The method according to claim 19 wherein the endoscopic device is an apparatus comprising:

a UV light source for generating a light having at least one wavelength of UV light within the excitation spectrum of the fluorescing targeting construct ;

at least one transmitting light guide adapted at the proximal end for receiving light from the UV light source and transmitting the light to the distal end thereof so as to irradiate a field of vision with the UV light;

at least one receiving light guide for receiving fluorescence from the field of vision at the distal end thereof and transmitting the fluorescence to the proximal end thereof; and

an eyepiece adapted to receive the fluorescence from the proximal end of the receiving light guide and to provide a visual image of the fluorescence for direct viewing by an operator.

27. The method according to claim 19 wherein a source of the UV light is located outside of the body of the subject.

28. The method according to claim 19 wherein the endoscopic device is selected from the group consisting of a laparoscope, a bronchoscope, a thoracoscope, an endoscope, a colonoscope, a cystoscope, a culposcope, an angioscope, and an arthroscope.

29. The method according to claim 1 wherein the viewing is for monitoring the course of the disease state.

30. The method according to claim 1 wherein the viewing identifies the tumor tissue for surgical intervention.

31. The method according to claim 1 wherein the subject is located in an operating room and the conditions that substantially eliminate the extraneous light comprise substantially darkening lights other than the UV light in the operating room.

32. The method according to claim 1 wherein the method further comprises surgically removing at least a portion of the tissue associated with the disease state.

33. The method according to claim 32 wherein the removing is by a device selected from the group consisting of a laparoscope, a bronchoscope, a thoracoscope, an endoscope, a colonoscope, a cystoscope, a culposcope, an angioscope, and an arthroscope.

34. The method according to claim 1 further comprising administering to the subject at least one supplemental fluorescing targeting construct that binds to the targeting construct to enhance the fluorescence.

35. The method according to claim 34 wherein the at least one supplemental fluorescing targeting construct comprises a monoclonal antibody, or biologically active fragment thereof.

36. The method according to claim 35 wherein the fragment is selected from the group consisting of Fab, Fab', (Fab')<sub>2</sub>, Fv, and single chain antibody fragments.

37. The method according to claim 1 further comprising administering to the subject a supplemental fluorescing targeting construct that specifically associates with normal tissue in the *in vivo* body part, wherein fluorescence from the supplemental fluorescing targeting construct in response to the UV light is a different  
5 color than that from the targeting construct, and wherein the different color distinguishes the tumor tissue from the normal tissue in the body part.

38. The method according to claim 2 wherein the fluorescing targeting construct further comprises a linker moiety for attaching the ligand moiety to the fluorescing moiety.

39. The method according to claim 38 wherein the linker moiety is a heterobifunctional crosslinker.

40. The method according to claim 39 wherein the heterobifunctional cross-linker is selected from the group consisting of N-succinimidyl (4-iodoacetyl)-aminobenzoate; sulfosuccinimidyl (4-iodoacetyl)-aminobenzoate; 4-succinimidyl-oxycarbonyl- $\alpha$ -(2-pyridyldithio) toluene ; sulfosuccinimidyl-6-[ $\alpha$ -methyl- $\alpha$ -(pyridyldithiol)-toluamido] hexanoate; N-succinimidyl-3-(-2-pyridyldithio)-propionate; succinimidyl-6-[3-(-2-pyridyldithio)-propionamido] hexanoate;  
5 sulfosuccinimidyl-6-[3-(-2-pyridyldithio)-propionamido] hexanoate; 3-(2-pyridyldithio)-propionyl hydrazide, Ellman's reagent, and dichlorotriazinic acid.

41. The method according to claim 38 wherein the linker moiety is a peptide having from about 2 to about 60 amino acid residues.

42. The method according to claim 41 wherein the peptide contains amino acid residues having a sequence selected from the group consisting of SEQ ID NOS: 1-10.

43. The method according to claim 2 wherein the UV-sensitive fluorescing moiety is selected from the group consisting of rhodamine, fluorescein, and tetracycline.

44. The method of claim 1 wherein the administering is by a method selected from the group consisting of topically, intraarticularly, intracisternally, intraocularly, intraventricularly, intrathecally, intravenously, intramuscularly, intravascularly, intercavitarily, intraperitoneally, intradermally, and by a combination  
5 of any two or more thereof.

45. The method according to claim 1 wherein the administering is by local injection.

46. The method according to claim 1 wherein the administering is systemic.

47. A method according to claim 1, further comprising removing at least a portion of the tumor tissue wherein the viewing of the fluorescence and the removing of the tumor tissue are performed substantially contemporaneously.

48. The method according to claim 47 wherein the targeting construct further comprises a biologically compatible UV-sensitive fluorescing moiety linked to the tumor-avid moiety.

49. The method according to claim 48 wherein the tumor-avid moiety is a hormone, deoxyglucose, somatostatin, a somatostatin receptor-binding peptide, or a combination of any two or more thereof.



51. A method for *in vivo* identification of tumor cells associated with a disease state in a subject in need thereof, said method comprising:

contacting samples of the tumor cells obtained from the subject *in vitro* with a plurality of detectably labeled antibodies and/or tumor-avid compounds,

determining which of the antibodies and/or tumor-avid compounds bind to or are taken up by the sample cells,

administering to the subject a diagnostically effective amount of one or more biologically compatible fluorescing targeting constructs, each comprising as ligand moiety at least one of the antibodies and/or tumor-avid compounds determined to bind to and/or be taken up by the sample cells so as to allow the fluorescing targeting construct to bind to and/or be taken up selectively *in vivo* by tumor tissue,

irradiating an *in vivo* body part of the subject suspected of containing the tumor tissue with UV light having at least one wavelength in the excitation spectrum of the targeting construct under conditions that substantially eliminate extraneous light to the *in vivo* body part, and

directly viewing fluorescence emanating from the fluorescing targeting construct bound to or taken up by the tumor tissue so as to determine the location and/or surface area of the tumor tissue in the *in vivo* body part.

**THIS PAGE BLANK (00PT0)**